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Antimicrobial Properties of a Traditionally and Specially Prepared Oil Complex: *NigellaSativa* Seed Oil, Rosemary Oil, and Olive Oil

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ABSTRACT

Herbal medicine also called botanical or phyto-medicine refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease. This study was focused on a traditionally prepared and used a product consist of an oil complex (olive oil, *Nigella sativa* seed oil, rosemary oil); which were researched antimicrobial effect of the product alone, and each oil by disc diffusion methods. Gram-positive and -negative bacteria and yeasts were employed in the susceptibility tests. The antibiotics of imipenem and vancomycin were exploited as positive controls. While there wasn't effect on Gram-negative bacteria, strong antimicrobial effects were observed on Gram-positive bacteria and yeasts. *Nigella sativa* seed oil was the most effective. There wasn't antagonistic and/or synergetic effect for the oil complex product. Although essential oils may be assigned the same name, they may differ widely as antimicrobial agents due to the extraction method used, which may explain the distinctive antimicrobial results reported in different studies. This was the first study to investigate the antibacterial and antifungal effects of this herbal medicine.

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Introduction

Plants carry out important biological functions such as self-protection against attack from bacteria, fungi, insects, and herbivorous mammals. Many such functions are mediated via a wide variety of chemical compounds, many of which have beneficial effects on human health when consumed and can be used effectively to treat human diseases. The use of plants as therapeutic agents in medicine predates written human history. Ethnobotany, the study of traditional human uses of plants, can be informative for the discovery of future medicines Elumalai and Chinna (2012), Fabricant and Farnsworth (2001), Lai and Roy (2004), and Gupta et al. (2018) indicated these issues in their studies. Treating diseases with herbs is common in non-industrialised countries, where herbs can be purchased more affordably than expensive modern pharmaceuticals (Elumalai and Chinna, 2012). With recent scientific evidence for the effectiveness of herbal medicines, applications of herbal medicines have become increasingly more common. In addition to searching for new medicinal herbs, many scientists are exploring new uses for existing traditional herbs. In this respect, many researchers have focused on a traditionally used herbal medicine product. With this study, we have focused on a product which consists of a complex of essential oils prepared traditionally and used especially in Middle and Eastern Anatolia for centuries. This traditionally used product composed of three main oil derived from olive oil, *Nigella sativa* seed oil, and rosemary oil. This complex of essential oils is traditionally used as a nasal dropin patient with sinusitis, one drop in each nostril for 1–3 days. We investigated the antibacterial and antifungal effects of this traditional herbal medicine. Despite the information available regarding the three essential oils individually, we have found no information on the antibacterial activity of this complex as a whole. This study is the first to investigate the antibacterial and antifungal effects of this oil complex.

Materials and Methods

Essential oils

The product and three essential oils were obtained from NBV İlaç, Doğal Ted. Ürün Koz. Gıda ve Mad. Paz. San. Tic. LTD. ŞTİ. (Konya, Turkey), producer of the plant essential oils and the herbal medicine. The quality of the oils was >98.5%. These were dissolved in dimethylsulfoxide (DMSO) at a 1:1 ratio.

Test organisms

We tested Gram-negative and Gram-positive standard strains, fungi, and clinical isolates, which were identified by the conventional and automated identification system, Vitek 2. The Gram-negative standard strains were *Escherichia coli* (ATCC 25922), *Klebsiellae pneumoniae* (ATCC 15380), *Pseudomonas aeruginosa* (ATCC 10145), and *Proteus vulgaris* (ATCC 7002); the Gram-positive standard strains were *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212); and the standard fungi were *Candida albicans* (ATCC 10231) and *Candida tropicalis* (ATCC 13803). All clinical isolates were obtained from a culture stock library at the Clinic Diagnostic Laboratory of Medical Microbiology Department of our University Hospital.

Antimicrobial assay

To screen for the antibacterial and antifungal effects of the oil complex product and three essential oils, the disc diffusion method was employed as described previously by Agaoglu et al. (2005), Prabuseenivasan et al. (2006), Stefanovic et al. (2009), and Erturk et al. (2010), with little modification. Screening was performed using a 16–18 h culture at 36±1°C in 10 mL Mueller-Hinton broth. The cultures were adjusted to approximately 10^5 CFU/mL with sterile saline solution; 500 µL suspensions were spread over Mueller-Hinton agar plates (94 mm × 16 mm) (Sigma-Aldrich) using a sterile cotton swab to ensure uniform microbial growth. Mueller-Hinton agar plates with 5% sheep blood in media were used only with the streptococcus species, *S. pyogenes* and *S. pneumoniae*. Two Mueller-Hinton agar plates were employed for each of the bacterial and fungal samples. Under aseptic conditions, we used five blank discs (CT 998 B; Oxoid) and one positive control disc (30 mg vancomycin; BD-BBL) for Gram-positive bacteria, and 10 mg imipenem (BD-BBL) for Gram-negative bacteria. Blank discs were placed on the agar surface, arranged as follows: three discs for the three essential oils (olive oil, *Nigella sativa* seed oil, and rosemary oil), one disc for vehicle control (negative control), and one disc for the essential oil complex product. The three essential oils were examined on one Mueller-Hinton agar plate, and the positive and negative controls and the essential oil complex product were examined on the other Mueller-Hinton agar plate. After placing the discs, the agar plates with media were incubated at room temperature for 5 min to attach the discs on the media surface. Each essential oil (20 µL) was pipetted onto the related discs, and then the plates were placed in an incubator at 36±1°C for 18 h. These were incubated cover-side up for 1 h to allow diffusion of the oil, and then they were inverted for the remainder of the incubation.

Results and Discussion

The antibacterial activities of three essential oils and the oil complex product were tested against 13 bacterial species and 3 fungi species (Table 1).

Thirteen different bacteria genera (including 52 strains) and two different yeast genera (including 5 strains) were included in the evaluation. A sample was scored as positive when the zone of inhibition of the sample was greater than or equal to that of the positive control. No antibacterial effect was observed against Gram-negative bacteria; however, all Gram-positive bacteria and yeast showed sensitivity. The results showed that the main antimicrobial effect originated from *Nigella sativa* seed oil. Although each of the oils in the mixture displayed antimicrobial effects separately, the oil complex showed no synergetic effect. All vehicle controls (negative controls) showed no inhibition of growth.

Traditional medicine or complementary medicine has a long history of use in health maintenance, disease prevention, and treatment of chronic disease. In this respect, traditional medicine has great potential to improve health and wellness; however, it requires particular attention to avoid misunderstandings and inappropriate use that could be harmful to individuals. There is a growing trend in which people, with or without health problems, are taking a more proactive approach to their own health and seeking out different forms of self-care. Many of these individuals have an assumption that natural means safe. Almost one quarter of all modern therapeutic drugs originate from natural products, many of which were used first in a traditional medicine product. Therefore, traditional medicine is a resource for primary health care but also for innovation and discovery. Working together, traditional and modern medicine systems might produce beneficial harmony, taking advantage of the best features of each system and compensating for certain weaknesses of the other as mentioned by Basmacıoglu-Malayoglu et al. (2011), WHO traditional Strategy 2014-2023 (2013), Barnes (2003), Hassan et al. (2009), and Zhang (2018). This study examined the utility of a traditional complex in a modern medicine system by analysing the product's antimicrobial and antifungal effects.

Although the essential oils and the herbal medicine showed no antibacterial effects on Gram-negative bacteria, we found strong effects on Gram-positive bacteria and yeasts in the micro-organisms examined. The antibacterial effects on *Enterococcus faecalis*, *Enterococcus feacium*, and *Enterococcus gallinarum* originated from *Nigella sativa* seed oil. The antibacterial effects on *Streptococcus pyogenes* and *Streptococcus pneumonia* originated not only from the *Nigella sativa* seed oil alone, but also from olive oil and rosemary oil. These two essential oils alone appeared weaker than *Nigella sativa* seed oil, as the effect of olive oil showed a borderline effect that was less than that of the positive control. The antibacterial effects on the staphylococcus species *S. aureus*, *S. haemolyticus*, and *S. epidermidis* were as follows: olive oil had no effect on *S. aureus or S. haemolyticus* but had a slight effect against *S.epidermidis*. Rosemary oil had a weaker effect than *Nigella sativa* seed oil but a greater one than olive oil. Olive oil showed no effect against the yeasts *C. albicans*, *C. tropicalis*, and *C. neoformans*, whereas the other oils did, with *Nigella sativa* seed oil being the most effective.

Table 1 Antimicrobial activities of the essential oils and the oil complex product

Microorganism	Antimicrobial activity (inhibition zone, mm)			
	A	B	C	OCP
Gram-negative bacteria				
Escherichia coli ATCC 25922 and four clinical isolates	none	none	7.0 (±1)	5.0 (±1)
Pseudomonas aeruginosa ATCC 27833, ATCC 10145 and four clinical isolates	none	none	none	none
Acinetobacter baumannii: four clinical isolates	none	none	none	none
Klebsiella pneumonia: four clinical isolates	none	none	none	none
Proteus vulgaris (ATCC 7002) and four clinical isolates	none	none	none	none
Gram-positive bacteria				
Enterococcus faecalis (ATCC 29212) and four clinical isolates	none	19.3 (±0.2)	none	18.5 (±0.3)
Enterococcus faecium four clinical isolates (VRE)	none	20.1 (±0.2)	none	19.2 (±0.2)
Enterococcus gallinarum four clinical isolates (VRE)	none	20.4 (±0.1)	none	19.3 (±0.1)
Streptococcus pyogenes four clinical isolates	17.0 (±0.1)	46.8 (±0.2)	22.0 (±0.1)	45.3 (±0.3)
Streptococcus pneumonia four clinical isolates	17.0 (±0.1)	47.6 (±0.2)	22.0 (±0.1)	46.0 (±0.3)
Staphylococcus aureus ATCC 29213 and four clinical isolates	none	58.0 (±0.3)	12.0 (±0.1)	52.0 (±0.3)
Staphylococcus haemolyticus four clinical isolates	none	59.7 (±0.2)	12.4 (±0.2)	55.2 (±0.3)
Staphylococcus epidermidis four clinical isolates	12.0 (±0.2)	61.8 (±0.2)	23.2 (±0.1)	57.3 (±0.3)
Yeast				
Candida albicans ATCC 10231 and four clinical isolates	none	42.1 (±0.2)	15.3 (±0.1)	36.3 (±0.1)
Candida tropicalis (ATCC 13803) and four clinical isolates	none	42.0 (±0.3)	16.2 (±0.1)	38.0 (±0.1)
Cryptococcus neoformans one clinical isolate	none	39.1 (±0.1)	13.1 (±0.2)	35.3 (±0.1)
Enterobacteriaceae	IMP ≥23			
Acinetobacter baumannii	IMP: ≥22			
Pseudomonas aeruginosa	IMP: ≥19			
Enterococcus faecalis / faecium	VA[I]: ≥17			
S. pyogenes, S. pneumoniae	VA: ≥17			

Notes: A, olive oil; B, *Nigella sativa* seed oil; C, rosemary oil; OCP, the oil complex product; VRE, vancomycin-resistant enterobacteria; IMP, imipenem; VA, vancomycin; CLSI 2014 M100-S24

Viljoen et al. (2003) and Fu et al. (2007) had been indicated in their experiments; the main compounds found in rosemary essential oil are 1,8-cineole, α-pinene, and camphor, which have antibacterial and antifungal effects. Camphor and 1,8-cineole are reported to have antimicrobial effects, and camphor is reported to have antiseptic and decongestive effects (Viljoen et al., 2003). The topical use of camphor for fibrosis has also reported (Hasan et al., 2013). Viljoen et al. (2003), Khan (1999), Akgul et al. (1989), Carson and Riley (1995) and Shunying et al. (2005) indicated in their researches, cineole has antibacterial effects against Gram-positive samples but not against *P. aeruginosa* (Pattnaik et al., 1997). Rosemary oil has strong antibacterial effects, with consistent inhibition against Gram-negative and Gram-positive bacteria (Prabuseenivasan et al., 2006). In the present study, rosemary oil had antibacterial effects against *S. pyogenes*, *S. pneumonia*, *S. aureus*, *S. haemoliticus*, and *S. epidermidis*. It also had antifungal effects against *C. albicans*, *C. tropcalis*, and *C. neoformans*. Previously, cineole was found to have antifungal activity, especially against *C. albicans* (Viljoen et al., 2003).

Nigella sativa seed oil has many advantageous medical properties, and is universally called a panacea (Khan 1999). Notably, of the three oils, it showed the most effective antibacterial and antifungal in the present study, and had almost the same antibacterial and antifungal effects as the oil complex product, which included all three essential oils together with some supplements. Therefore, the concentration of *Nigella sativa* seed oil was reduced in the complex, but its effects alone nearly matched that of the complex, implying that it accounted for most of the overall effect. Previous studies managed by Hanafy and Hatem (1991), Morsi (2000), and Salman et al., (2008) that examined the antibacterial and antifungal effects of *Nigella sativa* seed oil showed different results against Gram-negative bacteria compared to our study. However, Hasan et al., (2013) detected lower antibacterial activity against Gram-negative than against Gram-positive bacteria, similar to our results. Medina et al., (2006), Medina et al., (2007), and Shan et al., (2013) indicated in their study that olive oil and olive leaf extracts have been used in traditional medicine for centuries because of their antimicrobial activity, which originates from phenolic compounds. In the present study, we observed a borderline antibacterial effect on *S. pyogenes* and *S. pneumonia* only.

An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition in the lipid layers of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable. Thus, leakage of the cell contents can occur. Although cells can tolerate a certain amount of leakage without loss of viability, extensive loss of cell contents or exit of critical molecules and ions leads to cell death (Burt, 2004). Essential oils or aromatic plant essences are volatile, and fragrant substances that have oily consistencies are frequently produced by plants. Such substances can be in liquid form at room temperature, although several are solid or resinous. Some substances show different colours ranging from pale yellow to emerald green and from blue to dark brownish-red. These substances can be synthesised by a variety of plant organs, i.e., buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, and bark, and are stored in secretory cells, cavities, canals, epidermic cells, and glandular trichomes. Essential oils from different parts of an aromatic plant can be extracted by various techniques, including water or steam distillation, solvent extraction, expression under pressure, and supercritical and subcritical water extractions (Bassolel and Juliani, 2012). Therefore, although essential oils may be assigned the same name, they may differ widely as antimicrobial agents due to the extraction method used. This difference in extraction method may explain the distinctive antimicrobial results reported in different studies.

In conclusion, the oil complex had strong antimicrobial effects on standard and clinically isolated micro-organisms, but no synergistic effect was observed, and *Nigella sativa* seed oil was mainly responsible for the antibacterial activity of the complex. In fact, *Nigella sativa* seed oil alone slightly outperformed the complex. This could be explained by the reduced amount of *Nigella sativa* seed oil in the product.

This is the first study to examine this oil complex product, which is still used in traditional medicine. In an ideal world, traditional medicine would be an option and choice, offered by a well-functioning, people-centred health system that balances curative services with preventive care. The challenge is to appropriately include traditional medicine in an integrated health system, to help all practitioners understand its unique and valuable contribution, and to educate consumers about what it can and cannot do. In other words, this rich resource and cultural heritage must be modernised and evaluated with respect to accepted Western practises. Further studies are needed to understand the medicinal properties of this composition.

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